

## CLAIMS

1. A method of preparing a recombinant allergen derived from a naturally occurring allergen, wherein  
5 specific IgE binding to the recombinant allergen is reduced compared to the IgE binding to said naturally occurring allergen, which method comprises:
  - a) identifying amino acid residues in said naturally occurring allergen which are conserved with more than 70%  
10 identity in all of the known homologous proteins within the taxonomic order from which said naturally occurring allergen originates;
  - b) defining at least one patch of said conserved amino acid residues which patch (i) is connected over at least 400 Å<sup>2</sup> of the surface of the three-dimensional structure of said naturally occurring allergen molecule; and (ii) comprises at least one B cell epitope, and wherein (iii) said conserved amino acid residues have a solvent accessibility of at least 20%; and  
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  - c) substituting at least one of said identified amino acid residues in said defined at least one patch with a non-conservative amino acid residue,  
20 wherein the α-carbon backbone tertiary structure of the recombinant allergen is essentially preserved as compared with the α-carbon backbone tertiary structure of said naturally occurring allergen.  
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2. The method of claim 1, wherein the amino acid residues identified in step (a) are 90% or more conserved.  
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3. The method of claim 1, which method further comprises:
  - b') ranking said conserved amino acid residues within said at least one patch in order of percent  
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solvent accessibility; and

b'') selecting said at least one amino acid residue for substitution in step (c) from the amino acid residues having a solvent accessibility of 20-80%.

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4. The method of claim 1, wherein the percent solvent accessibility is in a range from 30-80%.

5. The method of claim 1 in which the average root  
10 mean square of the atomic coordinates of the  $\alpha$ -carbon backbone tertiary structure of the recombinant allergen deviates from the average root mean square of the atomic coordinates of the  $\alpha$ -carbon backbone tertiary structure of said naturally occurring allergen by less than 2 $\text{\AA}$ .

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6. The method of claim 1, wherein the amino acid residue substitution in step (c) is performed using site-directed mutagenesis.

20 7. The method of claim 1, wherein said at least one amino acid residue being substituted is a polar amino acid and said non-conservative amino acid is a polar amino acid.

25 8. The method of claim 1, wherein said at least one amino acid residue being substituted is a non-polar amino acid and said non-conservative amino acid is a non-polar amino acid.

30 9. The method of claim 1, wherein said naturally occurring allergen is an inhalation allergen or venom allergen.

35 10. The method of claim 1, wherein said naturally occurring allergen is a tree pollen allergen, grass

pollen allergen, weed pollen allergen, herb pollen allergen, fungal allergen, dust mite allergen, animal allergen or insect allergen.

5        11. The method of claim 10 wherein

(a) said tree pollen allergen is from a tree taxonomically belonging to *Fagales*, *Oleales*, or *Pineales*;

(b) said grass pollen allergen is from a grass taxonomically belonging to *Poales*;

10        (c) said herb pollen allergen is from an herb taxonomically belonging to *Aasterales*;

(d) said fungal allergen is from a fungus taxonomically belonging to *Alternaria* or *Cladosporium*;

15        (e) said dust mite allergen is from a dust mite taxonomically belonging to *Dermatophagoides*;

(f) said animal allergen is from an animal taxonomically belonging to *Felis*, *Canis*, or *Equus*;

(g) said insect allergen is from an insect taxonomically belonging to *Blatella*, *Periplaneta*, or  
20        *Hymenoptera*;

(h) said weed pollen allergen is from a weed taxonomically belonging to *Urticales*.

12. The method of claim 11 wherein

25        (a) said tree taxonomically belonging to *Fagales* belongs taxonomically to *Betula*, *Alnus*, *Corylus*, or *Carpinus*;

(b) said tree taxonomically belonging to *Oleales* belongs taxonomically to *Olea*;

30        (c) said tree taxonomically belonging to *Pineales* belongs taxonomically to *Cryptomeria*;

(d) said grass taxonomically belonging to *Poales* belongs taxonomically to *Cynodon*, *Dactylis*, *Festuca*, *Holcus*, *Lolium*, *Phleum*, *Poa*, *Secale* or *Sorghum*;

35        (e) said herb taxonomically belonging to *Aasterales*

belongs taxonomically to *Ambrosia*, *Artemicia*, or *Urticales*;

(f) said fungus taxonomically belonging to *Alternaria* belongs taxonomically to *Alternata*;

5 (g) said fungus taxonomically belonging to *Cladosporium* belongs taxonomically to *herbarium*;

(h) said dust mite belonging taxonomically belonging to *Dermatophagoides* belongs taxonomically to *Farinae* or *Pteronyssinus*;

10 (i) said animal taxonomically belonging to *Felis* belongs taxonomically to *Domesticus*;

(j) said animal taxonomically belonging to *Canis* belongs taxonomically to *familiaris*;

15 (k) said animal taxonomically belonging to *Equus* belongs taxonomically to *caballus*;

(l) said insect taxonomically belonging to *Blatella* belongs taxonomically to *Germanica*;

(m) said insect taxonomically belonging to *Periplaneta* belongs taxonomically to *americana*;

20 (n) said insect taxonomically belonging to *Hymenoptera* belongs taxonomically to *Vespidae*, *Apidae*; and

(o) said weed taxonomically belonging to *Urticales* belongs taxonomically to *Parietaria*.

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13. A recombinant allergen derived from a naturally occurring allergen, wherein at least one solvent-accessible amino acid which is essentially conserved with more than 70 % identity in all of the known homologous

30 proteins within the taxonomic order from which said naturally occurring allergen originates, is substituted with an amino acid residue that is not conserved in homologous naturally-occurring allergens within said taxonomic order, wherein the  $\alpha$ -carbon backbone tertiary  
35 structure of the recombinant allergen is conserved as

compared with said naturally occurring allergen, and wherein specific IgE binding to the mutant allergen is reduced compared to IgE binding to said naturally-occurring allergen.

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14. The recombinant allergen of claim 13, wherein said at least one solvent-accessible amino acid residue has a solvent accessibility of 20-80%.

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15. The recombinant allergen of claim 13, in which between 1 and 5 solvent-accessible amino acid residues per at least 400 $\text{\AA}^2$ , are substituted.

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16. The recombinant allergen of claim 13, wherein the average root mean square deviation of the atomic coordinates comparing the  $\alpha$ -carbon backbone tertiary structures of said recombinant and said naturally occurring allergen is less than 2 $\text{\AA}$ .

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17. The recombinant allergen of claim 13, wherein the specific IgE binding to said recombinant allergen is reduced by at least 5-10%.

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18. The recombinant allergen of claim 17, wherein the specific IgE binding to said recombinant allergen is reduced by at least 10%.

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19. The recombinant allergen of claim 13, wherein said naturally occurring allergen is an inhalation allergen or venom allergen.

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20. The recombinant allergen of claim 13, wherein said naturally occurring allergen is a tree pollen allergen, grass pollen allergen, weed pollen allergen, herb pollen allergen, fungal allergen, dust mite

allergen, animal allergen or insect allergen.

21. The recombinant allergen of claim 20 wherein
  - (a) said tree pollen allergen is from a tree taxonomically belonging to *Fagales*, *Oleales*, or *Pineales*;
  - (b) said grass pollen allergen is from a grass taxonomically belonging to *Poales*;
  - (c) said herb pollen allergen is from an herb taxonomically belonging to *Aasterales*;
  - (d) said fungal allergen is from a fungus taxonomically belonging to *Alternaria* or *Cladosporium*;
  - (e) said dust mite allergen is from a dust mite taxonomically belonging to *Dermatophagoides*;
  - (f) said animal allergen is from an animal taxonomically belonging to *Felis*, *Canis*, or *Equus*;
  - (g) said insect allergen is from an insect taxonomically belonging to *Blatella*, *Periplaneta*, or *Hymenoptera*;
  - (h) said weed pollen allergen is from a weed taxonomically belonging to *Urticales*.

22. The recombinant allergen of claim 21 wherein
  - (a) said tree taxonomically belonging to *Fagales* belongs taxonomically to *Betula*, *Alnus*, *Corylus*, or *Carpinus*;
  - (b) said tree taxonomically belonging to *Oleales* belongs taxonomically to *Olea*;
  - (c) said tree taxonomically belonging to *Pineales* belongs taxonomically to *Cryptomeria*;
  - (d) said grass taxonomically belonging to *Poales* belongs taxonomically to *Cynodon*, *Dactylis*, *Festuca*, *Holcus*, *Lolium*, *Phleum*, *Poa*, *Secale* or *Sorghum*;
  - (e) said herb taxonomically belonging to *Aasterales* belongs taxonomically to *Ambrosia*, *Artemicia*, or *Urticales*;

- (f) said fungus taxonomically belonging to *Alternaria* belongs taxonomically to *Alternata*;
- (g) said fungus taxonomically belonging to *Cladosporium* belongs taxonomically to *herbarium*;
- 5 (h) said dust mite belonging taxonomically belonging to *Dermatophagoides* belongs taxonomically to *Farinae* or *Pteronyssinus*;
- (i) said animal taxonomically belonging to *Felis* belongs taxonomically to *Domesticus*;
- 10 (j) said animal taxonomically belonging to *Canis* belongs taxonomically to *familiaris*;
- (k) said animal taxonomically belonging to *Equus* belongs taxonomically to *caballus*;
- (l) said insect taxonomically belonging to *Blatella*
- 15 belongs taxonomically to *Germanica*;
- (m) said insect taxonomically belonging to *Periplaneta* belongs taxonomically to *americana*;
- (n) said insect taxonomically belonging to *Hymenoptera* belongs taxonomically to *Vespidae, Apidae*;
- 20 and
- (o) said weed taxonomically belonging to *Urticales* belongs taxonomically to *Parietaria*.

23. The recombinant allergen of claim 20, wherein  
25 the recombinant allergen is derived from *Ves v 5*.

24. The recombinant allergen of claim 23, wherein  
the allergen has one or more amino acid substitutions  
selected from the group consisting of:  
30 i) Ala at position 72 of SEQ ID NO: 39; and  
ii) Ala at position 96 of SEQ ID NO: 39.

25. The recombinant allergen of claim 20, wherein  
the recombinant allergen is derived from *Bet v 1*.

26. The recombinant allergen of claim 25, wherein the allergen has one or more amino acid substitutions selected from the group consisting of:

- (i) Pro at position 10 of SEQ ID NO: 37;
- 5 (ii) Gly at position 25 of SEQ ID NO: 37;
- (iii) Thr at position 28 of SEQ ID NO: 37, Gln at position 32 of SEQ ID NO: 37;
- (iv) Ser at position 45 of SEQ ID NO: 37;
- 10 (v) Ser at position 47 of SEQ ID NO: 37;
- (vi) Asn at position 55 of SEQ ID NO: 37;
- (vii) Ala at position 77 of SEQ ID NO: 37;
- (viii) Gly at position 108 of SEQ ID NO: 37;

and

- (ix) Thr at position 28 of SEQ ID NO: 37, Gln at position 32 of SEQ ID NO: 37, Ser at position 45 of SEQ ID NO: 37, and Gly at position 108 of SEQ ID NO: 37.

27. The recombinant allergen of claim 13, which is produced by a method comprising:

- 20 a) identifying amino acid residues in said naturally occurring allergen which are conserved with more than 70% identity in all of the known homologous proteins within the taxonomic order from which said naturally occurring allergen originates;
- 25 b) defining at least one patch of said conserved amino acid residues which patch (i) is connected over at least 400 Å<sup>2</sup> of the surface of the three-dimensional structure of said naturally occurring allergen molecule; and (ii) comprises at least one B cell epitope, and
- 30 wherein (iii) said conserved amino acids have a solvent accessibility of at least 20%; and

c) substituting at least one of said identified amino acid residues in said defined at least one patch with a non-conservative amino acid residue.

5        28. A pharmaceutical composition comprising a recombinant allergen prepared according to claim 1 and a pharmaceutically acceptable carrier.

10      29. A pharmaceutical composition comprising the recombinant allergen according to claim 13 and a pharmaceutically acceptable carrier.

15      30. A method of generating an immune response in a subject, which method comprises administering to the subject at least one recombinant allergen prepared according to claim 1 or a pharmaceutically acceptable composition comprising said at least one recombinant allergen.

20      31. A method of generating an immune response in a subject, which method comprises administering to the subject at least one recombinant allergen according to claim 13 or a pharmaceutically acceptable composition comprising said at least one recombinant allergen.

25      32. A method of vaccination or treatment of a subject, which method comprises administering to the subject at least one recombinant allergen prepared according to claim 1 or a pharmaceutically acceptable composition comprising said at least one recombinant allergen.

30      33. A method of vaccination or treatment of a subject, which method comprises administering to the subject at least one recombinant allergen according to

claim 13 or a pharmaceutically acceptable composition comprising said at least one recombinant allergen.

34. A method of treatment, prevention or alleviation  
5 of allergic reactions in a subject, which method  
comprises administering to the subject at least one  
recombinant allergen prepared according to claim 1 or a  
pharmaceutically acceptable composition comprising said  
at least one recombinant allergen.

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35. A method of treatment, prevention or alleviation  
of allergic reactions in a subject, which method  
comprises administering to the subject at least one  
recombinant allergen according to claim 13 or a  
15 pharmaceutically acceptable composition comprising said  
at least one recombinant allergen.